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NOTES ON GEOGRAPHIC DISTRIBUTON

First record of Soybean Stem Fly *Melanagromyza sojae* (Zehntner, 1901) (Diptera, Agromyzidae) in Uruguay confirmed by DNA barcoding

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Abstract. Colloquially known as Soybean Stem Fly, *Melanagromyza sojae* (Zehntner, 1901) (Diptera, Agromyzidae) is an oligophagous pest of plants in the family Fabaceae, including Uruguay's biggest commodity crop, Soybean (*Glycine max* (L.) Merr.). To our knowledge, this is the first scientific record of *M. sojae* in Uruguay, and we confirm its identity through using DNA barcoding. Characteristic damage to host plants and immature stages of *M. sojae* were confirmed in Dolores and Colonia, Uruguay.

Key words. *Glycine max,* mtDNA COI, soybean stem minor, soybean pests, stem borer flies

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INTRODUCTION

Melanagromyza sojae (Zehntner, 1901) is a global oligophagous pest which attacks many plant species of the family Fabaceae, although Soybean (*Glycine max* (L.) Merr.) is its main host (Spencer 1990). Colloquially known as Soybean Stem Fly, *M. sojae* has been reported damaging Alfalfa (*Medicago sativa* L.), Common Bean (*Phaseolus vulgaris* L.), Mung Bean (*Vigna radiata* (L.) R. Wilczek), Pea (*Pisum sativum* L.), Sweet Clover (*Melilotus* sp. Mill.), Chickpea (*Cicer arietinum* L.), among other species (Murúa et al. 2020). *Melanagromyza sojae* is native to East Asia (Spencer 1973), but it has been recently reported in South America in Brazil (Arnemann et al. 2016), Bolivia (Vitorio et al. 2019), Paraguay (Guedes et al. 2017), and Argentina (Trossero et al. 2020; Vera et al. 2020). Mitochondrial DNA has revealed the possibility of multiple introduction pathways for each of these countries, linked to growth in international trade (Vitorio et al. 2021). *Melanagromyza sojae* is not only widely distributed in South America and Asia, but also in many countries of Africa, Australia, and Europe (Spencer 1973; Gil-Ortiz et al. 2010).

Adults of *M. sojae* are black with a metallic-green pattern and approximately 2–3 mm long, and they complete their life cycle in 16–26 days (Spencer 1990; Hirose and Moscardi 2012). Females have a high rate of oviposition laying 75–95 eggs per female (Jadhav 2013), and they lay their eggs endophytically (or on the underside surface of leaves); days later, larvae emerge and enter the stems of the plants through leaf veins and petioles (Vera et al. 2021). *Melanagromyza sojae* undergoes three larval stages (approximately 2.3, 2.5, and 2.9 days, respectively); in the initial state larvae are whitish, and by the final stage they turn light yellow, reaching a length of approximately 4 mm (Vitorio et al. 2019). Larvae bore stems, mining both upwards and downwards in the stem. Reddish-brown galleries are formed in which pupa and puparia develop (Spencer 1973). Adults escape through a hole previously gnawed through by a fully grown larva (Van der Goot 1930). The short lifecycle and high rate of oviposition enable infesting populations of *M. sojae* to complete at least four generations per crop cycle. Pozebon et al. (2020) estimated the population growth curve for *M. sojae* and calculated that an initial population of one male and one female would need around 3–4 generations to infest all Soybean plants in a 1 ha area (one per plant). By the end of the fifth generation, this initial population would have produced over 200 million descendants. The activity of this species is favored by warm temperatures, high rainfall, and high humidity (Brier and Charleston 2013).

This pest has potential to be highly destructive. Damage goes unnoticed, as it is confined to the stem pith, but larvae can bore (consume pith) up to 70% of the length of the Soybean stem (Singh 1990, 1992). Thus, there are reports of yield losses of 2–80%, the amount varying according to region, crop nutrition, Soybean cultivar, sowing date, management strategies employed, and the phenological stage of the crop

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during infestation (Talekar 1989; Jadhav et al. 2013; Guedes et al. 2015; Murúa et al. 2020). The most susceptible stage of Soybean to *M. sojae* damage is early vegetative stages, as it can cause significant plant death (Kambrekar et al. 2018; Vitorio et al. 2019). In Uruguay, *M. sojae* represents a high-risk pest due its potential harm to the Soybean crop, one of the most important commodity crops in the nation. However, damage potential of *M. sojae* on Soyabean in Uruguay has not yet been determined.

METHODS

Soybean plants with symptoms of die-back or wilt were randomly collected in affected fields in south-western Uruguay, in Dolores and Tarariras, departments of Soriano and Colonia (Figure 1), during the 2021–2022 crop season. A distribution map was created using R (R Core Team 2015). Larvae and pupae were found on soybean stems with characteristic feeding damage. The infected stems displayed internally reddish-brown galleries with a zig-zag pattern, where larvae, pupae, and/or empty puparia were found, as described by Czepak et al. (2018) and Curioletti et al. (2018). The specimens were morphologically identified by the anterior and posterior spiracles of larvae and pupae (Vitorio et al. 2019) and preserved in 95% alcohol for further molecular identification.

Total DNA was extracted from individual samples according to a standard CTAB method described by Doyle and Doyle (1987). In this case, the entire body of the larva was used for DNA extraction. The mitochondrial cytochrome c oxidase subunit I gene (COI) was amplified and sequenced in triplicate using primers LepFolF RKTCAACMAATCATAAAGATATTGG and COISG-R TAAACTTCTGGRTGWCCAAAAAATCA. The PCR mix contained approximately 200 ng of total cell DNA, 10 pmol of each primer, 200 µmol of each dNTP, 2.5 µl of PCR buffer (with 20 mmol MgCl2), 0.8 units Taq DNA polymerase (Thermo Fisher Scientific, USA), ultrapure water was added for a final volume of 25 µl. Temperature cycling was as follows: 95 °C (4 min), 40 cycles of 95 °C (50 s), 52 °C (50 s), 72 °C (50 s), and a final extension at 72 °C (5 min). PCR products were purified by the standard method described by Sambrook and Russell (2006) and sent to Macrogen Inc. (Seoul, Korea) sequencing service (http://www.macrogen.com/). The resulting sequences were analyzed using a sequence alignment editor (BioEdit v. 7.2.5; Hall 1999). The taxonomic affinities of our nucleotide sequences were identified using the Basic Local Alignment Search Tool, BLAST (Johnson et al. 2008) and the Barcode of Life Data Systems (Ratnasingham and Hebert 2007). The obtained sequence has been deposited in GenBank (accession number is OR496139). Our phylogenetic analysis included sequence data of the COI barcode region for 24 flies of the family Agromyzidae, among them 12 sequences

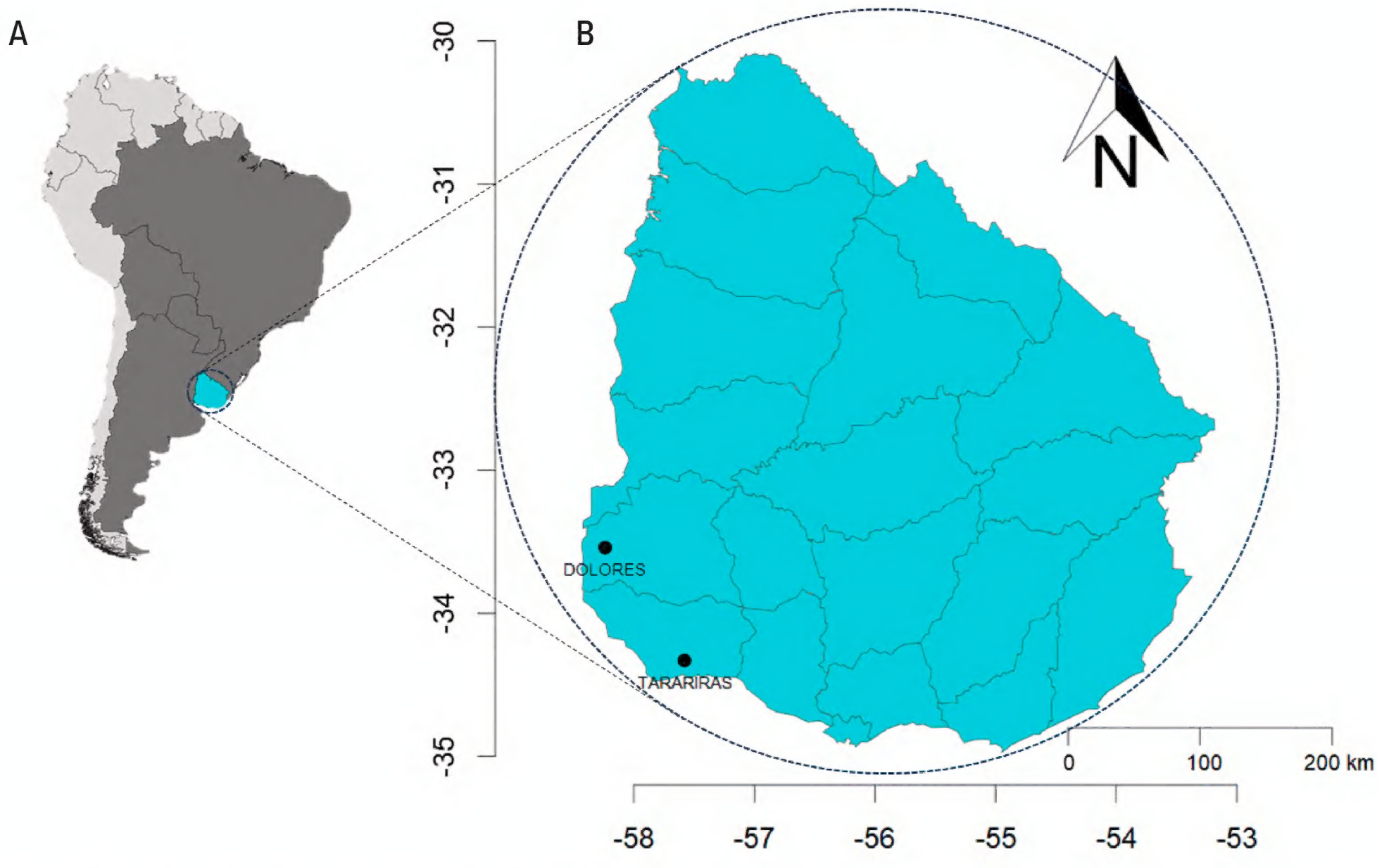

Figure 1. Distribution of *Melanagromyza sojae* in South America. **A.** Countries in dark gray where *M. sojae* has already been reported. **B.** Uruguay, with location of new records of *M. sojae*.

of *M. sojae* from different countries. The analysis was formed by the completely aligned sequences and evolutionary history was checked using the neighbor-joining method (Saitou and Nei 1987) in the MEGA 7 (Kumar et al. 2016). Finally, a phylogenetic tree was constructed using the maximum likelihood method and the Tamura-Nei model (Saitou and Nei 1987). The tree displaying the highest log likelihood (–8770.80) with percentages indicating the frequency of the associated taxa clustering together on the branches was chosen. The initial tree(s) for the heuristic search were generated using the neighbor-joining and BioNJ algorithms applied to pairwise-distance matrices estimated through the maximum-composite likelihood (MCL) approach.

RESULTS

Order Diptera
Family Agromyzidae

***Melanagromyza sojae* (Zehntner, 1901)**

New record. URUGUAY – **Soriano** • Dolores; −33.5400, −058.2386; 14.III.2022; Olivieri leg.; Soybean field trial; GenBank OR496139 (COI); specimen code LE17; 1 larva.

Identification. Soybean stems displayed distinctive, characteristic damage indicative of *M. sojae* infestation. The anterior spiracles of the larvae were as described for this insect species (Van der Goot 1930; Spencer 1973; Vitorio et al. 2019) (Figures 2, 3). The 2–3 mm long pupal stage also had well-separated posterior spiracles, which typically manifest as six raised pores arranged around a central truncated horn (Vitorio et al. 2019).

Three PCR products were successfully sequenced for the DNA barcode region of the COI gene, which had a sequence length of 658 bp with no evidence of stop codons or contamination. The three sequences were identical. Using the BOLD ID Engine, the comparison with the DNA barcode library resulted in a 99.7% pairwise nucleotide match with seven *M. sojae* sequences in BOLD, confirming our morphological identification. An NCBI BLAST analysis retrieved five *M. sojae* nucleotide sequences showing a 99.7% match to our OR496139 sequence.

The phylogenetic tree (Figure 3) shows that all 12 samples of *M. sojae*, originating from various countries, independently grouped together within a strongly supported clade, as evidenced by robust bootstrap values of 97% across all three trees. Notably, our specimen also falls within this clade, but demonstrating a closer genetic affinity to the Argentine haplotypes.

Figure 2. A. Soybean stem bored by *Melanagromyza sojae* larva. **B, C.** Larva of the *Melanagromyza sojae* feeding on pith of soybean stems.

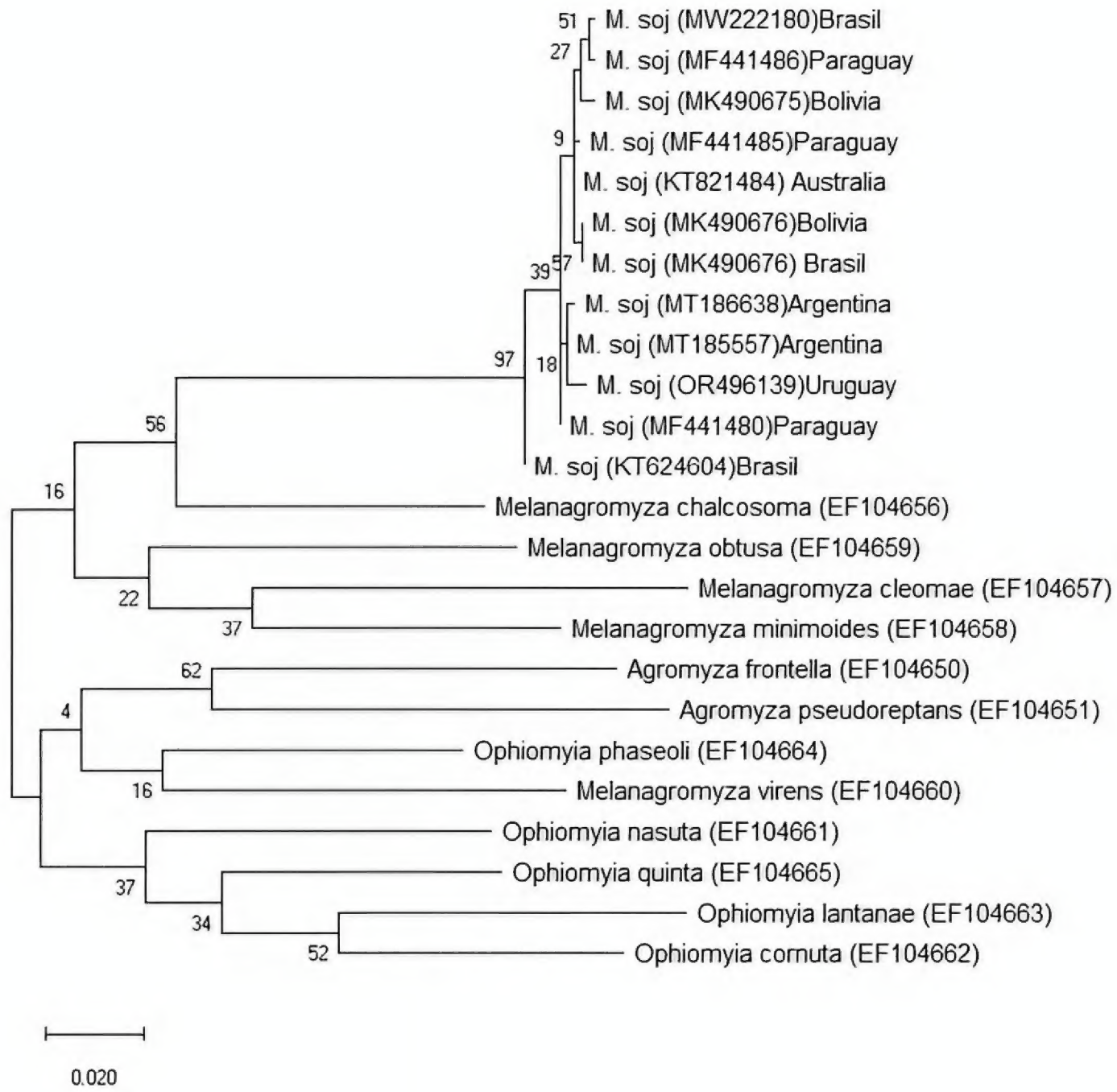

Figure 3. Phylogentic tree showing the maximum-likelihood method and Tamura-Nei model (Saitou and Nei 1987). The tree with the highest log likelihood (–8770.80) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths representing substitutions per site. This analysis involved 24 GenBank nucleotide sequences from the Agromyzidae family (accesion numbers specified on the tree).

DISCUSSION

Melanagromyza sojae is a harmful crop pest, it has been seen infesting 100% of Soybean plants in Argentine crops especially in late-sowing dates (Vitorio et al. 2019; Murúa et al. 2021). This fly species has been detected across diverse eco-climatic regions, which infers its potential to establish in Uruguay (Vitorio et al. 2019).

The delayed planting of Soybeans and the presence of volunteer Soybean plants in fields, along with the availability of alternative overwintering hosts such as Persian Clover (*Trifolium resupinatum* L.), allow *M. sojae* populations to endure winter conditions and pose a threat to summer crop production, as noted by Ferreira et al. (2020) elsewhere in South America. Persian Clover, as well as other legumes such as Alfalfa, is frequently used in pasture systems in Uruguay. These species potentially may act as alternative overwintering host plants for *M. sojae*, although further investigation is needed. Large areas of Alfalfa and Clovers are available year-round as potential legume hosts for *M. sojae* (Fadda et al. 2023). Depending on the year, a substantial portion (36–55%) of the Soybean area in Uruguay is sown late, typically immediately after the harvest of winter crops (MGAP-DIEA 2019, 2020, 2021, 2022). This practice highly favors infestations by *M. sojae* (Fadda et al. 2023).

The small size of the adult fly makes it difficult to detect in the field, and both chemical (e.g. Curioletti et al. 2018) and biological (e.g. Beche et al. 2018) control are unreliable because the larvae are hidden and protected inside the stems. In South America many registered insecticides are available for pest management in Soybean crops. However, none of them are registered for the control of insects of the order Diptera, such as of *M. sojae* (Curioletti et al. 2018). *Melanagromyza sojae* usually goes unnoticed in crops until plants begin to die. Dead Soybean plants are often misdiagnosed with diseases such as brown-stem rot caused by *Cadophora gregata* (Allington & D.W. Chamb.) W. Gams, or wilted plants are misdiagnosed with root diseases such as *Fusarium* root rot or charcoal rot, which is caused by species of *Fusarium* Link and *Macrophomina phaseolina* (Tassi) Goid. (Vera et al. 2020). This makes us highly concerned about the establishment of *M. sojae* in Uruguay.

Given its significance as a Soybean pest in various regions, including the Old World and more recently in Brazil, Paraguay, and Bolivia, there is an urgent need to enhance monitoring for *M. sojae* in Soybean-producing areas of Uruguay. Furthermore, studies on the ecology, biology, and economic effects of *M. sojae* in Uruguay are essential to inform future management strategies in this country. Our results confirm that our haplotype is the same one that was reported in Argentina (Arnemann et al. 2016; Guedes et al. 2017).

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ADDITIONAL INFORMATION

Conflict of interest

The authors declare that no competing interests exist.

Ethical statement

No ethical statement is reported.

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Author contributions

Conceptualization: Data curation: MG. Formal analysis: MG. Funding acquisition: XCS. Investigation: XCS, SS. Methodology: XCS, SS, MG, VO. Resources: XCS. Supervision: XCS. Project administration: XCS. Software: MG. Validation: XCS, SS, MG. Visualization: XCS, SS, MG. Writing – original draft: XC, SS. Writing – review and editing: XCS, SS, MG, VO.

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Data availability

All data that support the findings of this study are available in the main text, and all nucleotide sequences used from GenBank are specified in Figure 3.

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